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Cytosolic Calcium Wave Propagation Depends on Local Calcium Movement Inside Cardiac Sarcoplasmic Reticulum

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The propagation of cytosolic Ca waves in cardiac myocytes is thought to occur through Ca-induced Ca release (CICR) with Ca being released from the sarcoplasmic reticulum (SR) via ryanodine receptors (RyR) activating neighboring RyRs and propagating throughout the cell. This widely accepted paradigm has recently been challenged by the hypothesis that an intra-SR 'sensitization' Ca wave precedes the cytosolic Ca wave and primes RyRs for activation by CICR (Keller et al., Cardiovasc. Res., 2007). Here we tested the intra-SR sensitization wave hypothesis by direct simultaneous measurements of cytosolic ($[Ca]_i$) and intra-SR ($[Ca]_{SR}$) calcium signals during wave propagation in intact and permeabilized rabbit ventricular myocytes. $[Ca]_i$ and $[Ca]_{SR}$ were measured with the fluorescent probes rhod-2 and fluo-5N, respectively, using high-speed confocal imaging. Our data show that the cytosolic Ca wave front and the corresponding intra-SR Ca depletion wave is preceded by a transient elevation of $[Ca]_{SR}$. This transient elevation of $[Ca]_{SR}$ preceded the cytosolic wave front along the path of wave propagation and could be identified at individual release junctions with high-resolution imaging. Colliding waves that originated at separate subcellular locations annihilated as expected, however the preceding elevation of $[Ca]_{SR}$ of each wave resulted in a further increase of $[Ca]_{SR}$ at the site of collision. Increasing SR Ca load (beta-adrenergic stimulation) and SR Ca buffer capacity (with the exogenous Ca buffer ADA) enhanced Ca wave amplitude and $[Ca]_{SR}$ elevation preceding the wave. Furthermore, the cytosolic Ca wave front was preceded by a small transient decrease of $[Ca]_i$ that coincided with the transient elevation of $[Ca]_{SR}$ and possibly indicates the involvement of SERCA activity. These data suggest that local Ca movement inside the SR is a prerequisite for the propagation of spontaneous Ca waves in cardiac myocytes.

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Subcellular Structural Changes in Diabetic Cardiomyopathy and its Impact on Cardiac Cell Calcium Dynamics

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Diabetic cardiomyopathy is characterized by reduced cardiac output and is linked to subcellular level changes in cell structure and function. Although a number of studies have quantified the effect on protein distribution, Ca2+ handling and kinetics, the alterations in spatial organization of the major cell organelles have not been fully quantified before. We therefore studied organelle distribution in detail to study the effect of changes on, for example, calcium dynamics in an integrated manner. Cells from healthy and diabetic (following injection of STZ) Wistar rats were imaged under a transmission electron microscope. The spatial distribution of the mitochondria and myofibrils were analyzed in these images to determine a statistical model (using spatial statistics theory) that would adequately represent their characteristic distribution in cells from healthy versus diabetic animals. The model was calibrated using control and diabetic animal data such as shown in Fig. 1. This also allowed us to statistically quantify the differences in spatial organization. We present these structural differences and assess the influence of these changes on the spatial and temporal dynamics of cytosolic calcium during calcium-induced calcium release in an integrated 3D computational model.

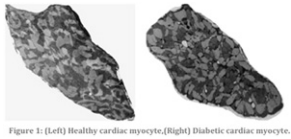

Figure 1: (Left) Healthy cardiac myocyte,(Right) Diabetic cardiac myocyte.

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Light Activated Insulin Release from Pancreatic Beta Cells

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The controlled release of insulin from pancreatic beta cells is a critical and complex process that is required for the maintenance of appropriate glucose levels in the blood. An efficient way to secrete naturally produced insulin could form the basis of new treatments for diabetes mellitus. Insulin release from beta cells is stimulated by the depolarization of the cell membrane. The light activated cationic Channelrhodopsin-2 based ion channel protein, known as chIEF, has been used in the past to depolarize neurons in culture. This protein was expressed in murine pancreatic beta cells. These cells were exposed to light at the excitation wavelength of the chIEF protein for ten mintues. The cells were exposed through different cycling protocols, in order to investigate the effect of rhythmic excitation. A novel FRET-based Ca2+ sensor was designed to attempt to monitor the dynamics of the Ca2+ gradients in response to this stimulation. It was found that specific patterns of light activation resulted in an increase in the amount of insulin secreted compared to cells which did not have the chIEF protein under the same light conditions. These results suggest that there is some oscillatory behavior in the Ca2+ depolarization of the beta cells, which can be tuned through adjusting the pattern of light excitation to produce controllable insulin release from pancreatic beta cells.

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Spatial-Temporal Dynamics of Collective Chemosensing

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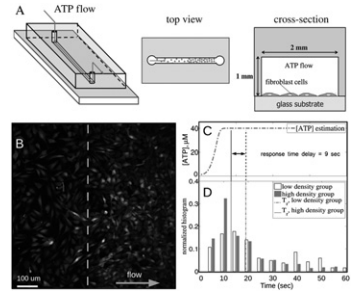
Although chemosensing by individual cells is stochastic, multi-cellular organisms exhibit highly regulated response to stimulations. The key elements to understand orders in chemosensing are cellular communications and the existence of pacemakers. To study the collective behaviors in chemosensing induced by these two factors, we studied spatial-temporal calcium dynamics of fibroblast cells in response to ATP in microfluidic devices. We found gap junction communications led to faster, more synchronized, and correlated responses compared to isolated cells. We demonstrated the existence of pacemakers and how they dictated the initial responses in the presence of gap junctions. By further studying the calcium dynamics of cells embedded in a thin hydrogel film, where cellular communications were only through diffusing molecules, we conclude that gap junctions are essential to generate prompt, synchronized and highly correlated responses. In addition, both communication channels lead to calcium oscillations following the elevation by external ATP in high density cell colonies. While the calcium oscillations associated with gap junctions were transient, the calcium oscillations in the hydrogel persisted more than 10 minutes and demonstrated rich structures in the Fourier spectrum. Further study is needed to understand the ATP-triggered collective behavior within the hydrogel.



525-Pos Board B311

Calcium Alternans in a Couplon Network Model of Ventricular Myocytes: Roles of Sarcoplasmic Reticulum Load

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Intracellular calcium (Ca) alternans of cardiac myocytes has been shown in many experimental studies, and the mechanisms remain incompletely understood. We recently developed a ''3R theory'' in which alternans arises as a result of the interactions of three critical properties: Randomness of Ca sparks; Recruitment of a Ca spark to its neighboring Ca release units (CRUs); and Refractoriness of the CRU. On the other hand, experimental studies have shown that sarcoplasmic reticulum (SR) Ca load plays important roles in the genesis of Ca alternans. In this study, we use computer simulation to study how SR Ca load and other physiological parameters, such as RyR sensitivity, SERCA pump, CRU spacing, Na-Ca exchange strength, L-type Ca conductance, etc., affect Ca alternans. Our model consists of 100 x 20 x 10 CRUs coupled via Ca diffusion in the cytoplasmic and SR space. Each CRU contains 100 RyRs with individual RyRs simulated stochastically. We developed a method to calculate the primary spark rate and the recruitment rate, and paced the model to periodically to elicit Ca alternans. We show that altering the physiological parameters not only directly change the 3 R's but also alters the SR Ca load (or the total Ca of the cell), and thus Ca alternans properties. However, higher SR Ca load causes more Ca leak which in turn causes RyRs to be more sensitive to cytosolic Ca, affecting primary spark rate and recruitment. Therefore, our present study shows that although the 3R theory does not include the SR load per se, the SR Ca load affects Ca alternans via its effects on the 3 R's.

Intracellular Channels

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Biophysical Properties of a Novel Cationic Channel in the Outer and Inner Membranes of Nuclei from Adult Skeletal Muscle Fibers

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In spite of some limited progress, the physiological role of cationic ion channels within the nuclear envelope remains largely unknown. Since biophysically distinct channels have been reported within the nuclear envelope of different cells, it is likely that nuclear membrane ion homeostasis is cell-type specific. Here we